ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

Phytonutrients, Minerals, and Organoleptic Properties of Bread Produced from Bicarbonate Pretreated African Yam Bean (*Sphenostylis stenocarpa*) Seed Coats Flour and Wheat Flour

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Abstract:- Industrial consumers are facing a pressure on their foreign currency reserves due to the importation of wheat flour for bread manufacturing. The study investigated the phytonutrient composition, mineral composition, and sensory characteristics of bread made from a combination of wheat flour and pre-treated seed coatings derived from African yam bean (Sphenostylis stenocarpa). The bread was prepared by blending African yam bean seed coat with wheat flour in ideal quantities ranging from 21.8 to 78.2g. The bread sample underwent evaluation for its phytonutrient and antioxidant content, including total phenol, ABTS, and total carotenoid. Additionally, the sample was analysed for minerals such as calcium, phosphorus, potassium, zinc, and salt, as well as anti-nutrient components like trypsin inhibitor, oxalate, phytate, and tannin. Descriptive sensory evaluation was carried out on the appearance, texture and odour. Data obtained were analyzed using Analysis of Variance (ANOVA) with the use of Statistical Analysis Software (SAS). The results obtained for the phytonutrients and antioxidants were phenol (0.87 to 3.48 mg/g) ABTS (0.001 to 0.004 Mmol/g), carotenoid (0.52 to 1.04 mg/g). Mineral results were calcium (37.46 to 44.28 ppm), phosphorus (24.16 to 28.89 ppm), potassium (57.44 to 65.04 p pm), zinc (1.50 to 1.66 ppm) and sodium (64.21 to 76.30 ppm). Anti-nutrient factors of Trypsin inhibitor (1.06 to 5.34 %), oxalate (0.01 to 0.17 mg/g), phytate (0.11 to 0.35 mg/g) and tannin (0.21 to 1.39 mg/g). The sensory assessment of the bread indicated that sample 506, which is made from a composite flour derived from the seed coat of African yam beans, met the acceptable standards.

Keywords:- *Wheat Flour, Seed Coat, Bread, Phytonutrient, Minerals, Sensory.*

I INTRODUCTION

The Triticum spp. genus, often known as wheat, holds great importance being the major grain utilised in the manufacturing of bread (Dewettinck et al., 2008). The role of gluten in the bread recipe is influenced by its unique proteins and their relatively larger concentration in comparison to other ingredients (Popov-Raljić et al., 2009; Demirkesen et al., 2014). Composite flour or flour blends can be produced by combining wheat with flour obtained from non-wheat sources, such as roots, tubers, legumes, or other raw materials. The utilisation of wheat and legume flour blends has demonstrated efficacy in the production of bread that exhibits high nutritional value and a substantial content of dietary fibre. Additionally, this combination leads to a decelerated degradation of starch and a reduced glycemic index. Moreover, the dough derived from this mixture demonstrates viscoelastic properties, and the bread product possesses satisfactory sensory attributes (Angioloni and Collar, 2012).

Legumes are highly valuable sources of protein, especially in Nigeria where a substantial majority of the population resides below the poverty line and has the financial means to purchase protein obtained from animals (Saka et al., 2019). The increasing consumer awareness of the nutritional and health benefits of legumes, namely African yam bean, has led to a surge in global demand for these legumes (Nedumaran et al., 2015).

Despite its underutilization, the processing of this legume is challenging owing to its tough shell or coat. However, several procedures including soaking, fermentation, and germination have been found to facilitate the removal of the hull and improve its processing, hence expanding its potential uses (Maphosa & Jideani, 2017). Dehulling is a fundamental technique used in the first stages of processing legumes once they have been harvested (Hatice, 2017). Approximately 85% of legumes in Nigeria are dehulled before consumption, resulting in a significant accumulation of seed coatings (Erdil, 2016).

The primary source of macronutrients such as fibers, minerals, and phytonutrients (substances that inhibit nutrient absorption) in legume seeds is their seed coverings (Duenas *et al.*, 2006, Rochfort and Panozzo, 2007).Seed coats are frequently disposed of as waste either during or following the processing stage. Nevertheless, it is possible to utilise

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

these waste products by incorporating them into wheat flour. According to Takahama and Hirota (2010) and Bansee et al. (2016), implementing this approach has the potential to mitigate the elevated glycemic index (GI) and the subsequent susceptibility to GI-related disorders, including celiac-related diseases. Tropical regions generally do not possess native wheat and instead rely on imports. The current situation is exerting pressure on the foreign exchange reserves of industrial users that depend on wheat as a primary source for flour manufacturing in the bread industry. This study aimed to investigate the phytonutrient composition and sensory characteristics of bread created from a combination of wheat flour and pre-treated seed coatings derived from African yam bean (Sphenostylis stenocarpa). The objective was to explore the feasibility of utilising composite flour derived from these beans in the manufacturing of bread.

II. MATERIALS AND METHODS

A. Source of Material

Sphenostylis stenocarpa seeds, commonly known as African yam beans, were obtained from Ago Aduloju Market located in Ado Ekiti, Ekiti State, Nigeria. Golden Penny wheat flour was obtained from a reputable shop located in Ado-Ekiti, Ekiti State, Nigeria. The analytical grades of all reagents were utilised.

B. Dehulling of African Yam Bean

The dehulling process was conducted following the methodology outlined by Offia et al. (2015). Every sample, weighing 2000 g, underwent a meticulous removal of any foreign substances and was then immersed in cold water (1:3 w/v) for a duration of 12 hours. In order to isolate the seed coats from the cotyledon, the seeds underwent a manual dehulling process.

C. Pre-Treatment of African Yam Bean Seed Coats

The pre-treatment of the seed coat was conducted following the methodology outlined by Adebowale et al. (2013), with minor adjustments made to the soaking medium and duration. The seed coats (dehulled) were immersed in a solution of 2% Sodium Bicarbonate for a duration of 1 hour. The pretreated seed coat was placed in a hot air oven (MFRS Unicorn INSTRUMENTS, INDIA) and dried at a temperature of 55 o C until it reached a constant weight. The dried seed coat underwent milling using a mixer grinder (Mixer grinder.MX-AC 210S, Panasonic, JAPAN) and subsequent sieving at a size of 100 µm. Prior to use, the flour was packaged in high-density polyethylene.

D. Production of Bread

The bread production process involved the formulation of an optimised composite flour with a ratio of 78.2 g (wheat) to 21.8 g (seed coat). The bread preparation procedure followed the methodology outlined by Cauvain (2015). A dough was prepared by combining 200 g of flour with measured ingredients, including 5 g of salt, 40 g of margarine, 20 g of yeast, and 60 g of granulated sugar, in 500 ml of water. The mixture was then stirred using a Kenwood mixer (Model A 907 D, Japan) for a duration of 5 minutes. The dough was subjected to initial proofing in a baking tin for 55 minutes at room temperature (25 °C), followed by a second proofing in a cabinet for 90 minutes at 30 °C, 85% relative humidity, and then cooked at 250 °C for 30 minutes. Prior to analysis, the bread was cooled to room temperature and stored in low density polyethylene.

E. Phytochemical and Antioxidant Determination

The determination of the sample's total phenolic content was conducted using the methodology outlined by Gopalakrishnan and Perumal (2019). A quantity of 0.2 grammes of the extract was combined with 2.5 millilitres of a 10% Folin-Ciocalteau's reagent and 2 millilitres of a 7.5% sodium carbonate solution. The mixture underwent incubation for a duration of 40 minutes at a temperature of 45 degrees Celsius. Subsequently, the absorbance was quantified at a wavelength of 700 nm using a UV-Visible spectrophotometer (JENWAY 6405 Model, UK and England).

The ABTS assay, as described by Nilima and Hande (2011), was utilised to assess the radical scavenging activity and total antioxidant activity. The process of decolorization using 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS.+). The experimental protocol entailed the production of ABTS.+ radical cation through the combination of a 7 mM ABTS stock solution with 2.45 mM potassium persulfate. The mixture was then incubated in the absence of light at ambient temperature for a duration of 12-16 hours until the reaction reached completion and the absorbance remained constant. The ABTS.+ solution was adjusted to an absorbance of 0.70 (± 0.02) by diluting it with water at ambient temperature. A solution of ABTS.+ with a predetermined volume was combined with a known volume of the test sample. The measurement of absorbance was conducted at a wavelength of 734 nm following a duration of 6 minutes. The calculation and plotting of the % inhibition of absorbance were performed to obtain the trolox equivalent antioxidant concentration (TEAC) by varying the concentration of both the standard and sample. The ABTS radical scavenging activity (%) is calculated by subtracting the absorbance of the control from the absorbance of the sample.

Reversed phase high-performance liquid chromatography (HPLC) was employed to analyse the total carotenoids. The Thermo ACCELA U-HPLC instrument, manufactured by Thermo Fisher Scientific in Bremen, Germany, was equipped with a quaternary pump, an online degasser, a column oven controller, and a photodiode array detector (PDA). The separation of carotenoids was performed using a reverse-phase C30 column with a 3 µm size (250 ¼ 4.6 mm) connected to a 20 ¼ 4.6 mm C30 guard column (YMC Co., Kyoto, Japan). The mobile phases used were (A) methanol/water (98:2, v/v), (B) methanol/water (95:5, v/v), and (C) tert-methyl butyl ether. The column employed a gradient elution method consisting of 80% A and 20% C at 0 minutes. This was followed by a linear gradient to 60% A and 40% C for 2.00 minutes, with a flow rate of 1.4 mL/min. At 2.01 minutes, the flow rate was increased to 1.00 mL/min, and the gradient was changed to

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

60% B and 40% C. Subsequently, a linear gradient was applied to 0% B and 100% C for 12 minutes, and the column returned to its initial conditions by 13.00 minutes. At an initial concentration of 80% A and 20% C, a re-equilibration process lasting 7.00 minutes was conducted. The temperature of the column was always maintained at 20°C. PDA was used to monitor the eluting peaks within the wavelength range of 250 to 700 nm. The quantification process was conducted utilising Xcalibur software (version 2.2), wherein the peak area was compared to standard reference curves.

F. Mineral Content Determination

The wet method, as outlined by Onwuka (2018), was employed to ascertain the mineral composition of the sample. The elemental content of calcium, iron, zinc, sodium, potassium, and phosphorus was measured using an Atomic Absorption Spectrophotometer (Model: Thermo Scientific S series; Type: S4 AA System; NC: 942340030042; Model GE; Serial No: GE 712354, Thermo Electron Corporation, USA). A quantity of one gramme from each sample was carefully measured and transferred into a 125 ml Erlenmeyer flask. Subsequently, 20 ml of the acid mixture, which consisted of 325 ml of concentrated nitric acid, 40 ml of perchloric acid, and 10 ml of sulfuric acid, was introduced into the flask. The content was subjected to gentle mixing and heating in a digester (Buchi Digestion unit K…424) at a moderate temperature within a fume hood. The heating process was continued until a dense white fume was observed. The heating process was sustained for a duration of 30 seconds, after which it was allowed to cool. Subsequently, 50 cc of distilled water was added. The solutions were passed through a filter paper and transferred into a 100 ml volumetric flask. From there, distilled water was added to reach the mark. The solutions obtained were measured using an Atomic Absorption Spectrophotometer. The equipment underwent calibration using established standards, namely standards for magnesium measuring 0.5 ppm, 1.5 ppm, 3.0 ppm, and 4.5 ppm. Ca standards: 5 parts per million (ppm), 15 ppm, and 30 ppm. The standards for potassium (K) are 2, 6, and 10 parts per million (ppm). The standards for iron (Fe) were set at 2, 4, and 10 parts per million (ppm), while the standards for manganese (Mn) were set at 2, 5, and 10 ppm. The samples were then analysed at their respective wavelengths. To determine the concentration of each mineral, a hollow cathode lamp and holders were fitted in the lamp compartment, matching to the specific mineral being studied. The magnesium dilution factor is 10,000, while the dilution factor for other minerals such as calcium, iron, potassium, sodium, and manganese is 100.

G. Anti-Nutrient Determination

The determination of phytate was conducted using the methodology outlined by Marolt et al. (2021). A 4 g sample was immersed in 100 ml of a 2% hydrochloric acid solution for a duration of 3 hours and then passed through Whatman No. 2 filter paper. Subsequently, a volume of 25 ml of the filtrate was transferred into a conical flask, followed by the addition of 5 ml of a 0.3% ammonium thiocyanate solution. Following this, 53.5% of distilled water was introduced, and the entire mixture was subjected to titration using a standard iron (III) chloride solution until a brownish yellow hue was observed for a duration of 5 minutes. The concentration of phytate in the sample was quantified as a percentage (%).

The quantitative analysis of tannin content was conducted using the methodology outlined byAtanassova and Christova-Bagdassarian (2009). Approximately 2 grammes of the sample was measured and placed into a sample vial. Subsequently, 10 millilitres of 70% aqueous acetone were added and thoroughly sealed. The bottle was placed in an ice bath shaker and agitated for 2 hours at a temperature of 30 degrees Celsius. After centrifugation, the solution was separated and the liquid portion was placed on ice. 0.2 ml was transferred into a test tube using a pipette, and then 0.8 ml of pure water was added. A standard solution of tannic acid was generated by combining 0.5 mg/ml of the stock with distilled water to reach a final volume of 1 ml. The sample and standard were treated with 0.5 ml of Folin Ciocalteu's reagent, followed by 2.5 ml of 20% Na2CO3. After vortexing the solution, it was incubated at room temperature for 40 minutes. The absorbance of the solution was measured at a wavelength of 725 nm. The sample's tannin content was determined using a conventional tannic acid curve.

The determination of oxalate was conducted using the methodology outlined by Liu et al. (2009). In a volumetric flask, 2 g of the material was subjected to digestion using 10 ml of 6 M HCl for a duration of one hour, resulting in a final volume of 250 ml. The concentration of NH4OH solution was used to modify the pH of the filtrate until the solution transitioned from a salmon pink hue to a pale yellow shade. Subsequently, the filtrate underwent treatment with a 10 ml solution of 5% CaCl2 in order to induce precipitation of the insoluble oxalate. The suspension underwent centrifugation at a speed of 2500 revolutions per minute (rpm). Subsequently, the supernatant was separated by decantation, precipitation, and full dissolution in 10 ml of a 20% (v/v) H2SO4 solution. The filtrate obtained from the dissolving in H2SO4 was adjusted to a final volume of 300 ml. A 125 ml portion of the filtrate was heated until it reached the boiling point. It was then titrated against a 0.05 M standardised KMnO4 solution until a light pink colour appeared. This colour lasted for approximately 30 seconds, after which the burette reading was recorded. The concentration of oxalate was determined based on the titre value. The redox reaction as a whole is:

Oxalate Equation:

$$2M_nO_4^- + 5C_2O_4^{2-} + 16H^+ \rightarrow 2Mn^{2+} + H_2O + 10CO_2$$

The approach outlined by Ezequiel et al. (2017) was utilised to assess the trypsin inhibitory activity. To evaluate the trypsin inhibitory action, we calculated the disparity in enzyme activity between the sample, which contained inhibitors, and the standard, which did not. The sample's optical density (OD) data were adjusted by subtracting the

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

standard OD value and then graphed versus the volume of crude extract. The extract volume, equivalent to half of the standard optical density (OD) value, was designated as the sample volume that resulted in 50% inhibition. A single Trypsin Inhibitory Unit (TIU) is defined as such (Sadasivam and Manickam, 2016). The estimation of the TIU and specific activity was conducted using the formulas proposed by Babu et al. (2012).

$$\text{Specific activity (TIU per mg protein)} = \frac{TIU\ per\ gram\ of\ seed}{mg\ of\ protein} \quad (1)$$

H. Sensory Evaluation

Descriptive mean score for sensory attributes (appearance, texture and odour) were evaluated by three different bakers. A 9-point Hedonic scale was utilised, with the following values: 9 = Extremely like, 8 = Very much like, 7 = Moderately like, 6 = Very little like, 5 = Neither like nor dislike, 4 = Very barely dislike, 3 = Moderately dislike, 2 = Very much dislike, 1 = Extremely dislike.

I. Statistical Analysis

Three sets of data were collected and analysed using Analysis of Variance (ANOVA) with the Statistical Analysis Software SAS (2009) for Microsoft Windows. The Duncan Multiple Range Test (DMRT) was employed to separate the means, and a significance level of $p \geq 0.05$ was used to determine the presence of a significant difference.The experiments were performed in duplicate.

III. RESULTS AND DISCUSSION

A. Phytochemical and Antioxidant Content of Bread from Optimized Composite Flour

The bread made with optimised composite flour had a phenolic content ranging from 0.87 to 3.48 mg/g. The bread samples exhibited a statistically significant increase ($p<0.05$) in their phenolic content. The findings of the study indicate that the incorporation of African yam bean seed coat flours into wheat flour resulted in a statistically significant increase ($p<0.05$) in the phenolic content of the bread. Among the different types of bread, the African yam bean composite bread exhibited the highest phenolic value of 3.87 mg/g, whereas the 100% wheat bread displayed the lowest phenol value of 0.87 mg/g. According to Huang et al. (2015), there exists a direct proportionality between the antioxidant activity and the total phenol content. There is a positive correlation between the phenolic content of a product and its antioxidant activity. The greater antioxidant activity values reported for the composite bread samples, as shown in Table 1, can be attributed to this observation. The phenolic values observed in the current investigation were determined to be lower than the previously reported range of 32.72 to 71.79 mg/g for loaves made from wheat-Bambara groundnut flour blends, as reported by Mashau et al. (2022).

The optimised bread samples exhibited ABTS values ranging from 0.001 to 0.004%. The bread produced using a composite flour formed from African yam bean seed coat had the greatest ABTS value (0.004%), which was found to be substantially different ($p<0.05$) from the 0.001% ABTS value seen in the bread sample manufactured from 100% wheat flour, which had the lowest ABTS value of 0.001%. The ABTS index is a valuable tool for quantifying the antioxidant capacity of various dietary items (Munteanu and Apetrei, 2021). Consuming a diet rich in antioxidants can potentially lower the likelihood of developing several ailments, such as heart disease and cancer. According to Biskup et al. (2013), antioxidants have the ability to eliminate free radicals from the cells of the body and mitigate or minimise the harm resulting from oxidation. The present study observed ABTS values that were below the range of 0.04 to 0.08 reported by Wahyono et al. (2020) for bread samples made from wheat-pumpkin flour blends.

The optimised bread samples and control had a total carotenoid content ranging from 0.52 to 1.04 mg/g. The inclusion of African yam bean seed coat flours resulted in a statistically significant ($p<0.05$) elevation in the carotenoid content. In terms of total carotenoid content, it was observed that the 100% wheat bread exhibited the lowest value of 0.52 mg/g, whilst the African yam bean seed coat bread displayed the greatest value of 1.04 mg/g. Carotenoids are essential constituents of food that contribute to its nutritional value and health benefits. Carotenoids have a pro-vitamin A effect that improves the immune system and reduces the risk of developing diseases (Kourouma et al., 2019). The present investigation observed carotenoid levels in bread made from Wheat, Bambara Groundnut, and Yellow Root Cassava Flours, which were found to be lower than the reported range of 5.14 to 12.21 mg/g by Okoye & Ezeugwu (2019). The increased carotene levels seen in the previous study conducted by Kourouma et al. (2019) can perhaps be ascribed to the inclusion of yellow root cassava flour in the samples, as it was biofortified with β-carotene.

Table 1. Phytochemical and Antioxidant content of Bread Samples from Optimized Composite Flour from African Yam Bean Seed Coat and 100% Wheat Flour

Sample	Phenol (mg GAE/g)	ABTS (Mmol/g)	Total Carotenoid (mg/g)
AYB	$3.48^a \pm 0.00$	$0.004^a \pm 0.00$	$1.04^a \pm 0.03$
CW	$0.87^b \pm 0.05$	$0.001^b \pm 0.00$	$0.52^b \pm 0.02$

Values are means ± standard deviation of duplicate determinations. Means with different superscripts along the same column are significantly different ($p<0.05$).

AYB- Bread sample containing 21.762 g of African yam bean seed coats and 78.238 g of Wheat flour

CW- Control made from 100% wheat flour

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

B. Mineral Composition of Bread Samples from Optimized Composite Flour

The Calcium values for bread samples from optimized composite flour and control ranged from 37.46 to 44.28 ppm. African yam bean seed coat composite bread had the highest calcium value (44.28 ppm) which was significantly different ($p<0.05$) from 37.46ppm recorded for100%wheat bread. African yam bean is rich in calcium as reported by Shodehinde *et al.,*(2022). According to the Food and Agriculture Organisation (FAO, 2007), the presently advised nutritional intake (RNI) for calcium is 600 mg per day for children and 1000 mg per day for adults. According to Weaver and Heaney (2006), calcium is a micronutrient that plays a crucial role in maintaining health and overall well-being. It is responsible for a wide range of biological functions within the human body. It functions as an intermediary for almost all biological processes, provides stability to numerous proteins, and in insufficient quantities is linked to a wide range of diseases and disorders. Insufficient calcium intake may result in the development of rickets or osteoporosis (James et al., 2015). According to Sánchez et al. (2019), the seed coat exhibited a greater concentration of calcium compared to the cotyledon. The present investigation observed calcium levels that exceeded the range of 19.13 to 25.24 ppm reported by Oyinloye et al. (2023) for bread derived from wheat, moringa, coriander, and amaranth.

The mean phosphorus values for both the optimised bread samples and the control group varied between 24.16 and 28.89 parts per million (ppm). The findings indicated that bread made from a composite flour derived from African yam bean seed coat had the greatest phosphorus content (28.89 ppm), which was considerably higher ($p<0.05$) compared to the control sample with a phosphorus content of 24.16 ppm. The bread made entirely from wheat flour exhibited the lowest phosphorus content, measuring at 24.16 ppm. Sufficient phosphorus is necessary for the maintenance of bodily functions and the generation of energy. It collaborates with calcium to uphold optimal dental and skeletal health (Mi and Ejeh, 2018). The present investigation observed phosphorus values that were lower than the range of 72.3 to 101.9ppm reported by Igbabul et al. (2014) for bread made from wheat, maize, and orange-fleshed sweet potato flours. The observed low phosphorus levels in this study may be attributed to the increased phosphorus content in the cotyledon compared to the seed coat, as reported by Chávez-Mendoza et al. (2019).

Potassium values for the optimized bread samples and control ranged from 57.44 to 65.04ppm. The potassium value waslower in the bread sample from 100% wheatflour having the lowest potassium value (57.44 ppm) whilebread from African yam bean seed coat composite flour have the highest potassium value (65.04ppm). The findings of the study indicate that the incorporation of African yam bean seed coat flour into wheat resulted in a statistically significant increase ($p<0.05$) in the potassium content of the bread samples.

Potassium regulates fluid equilibrium in the body and facilitates the transmission of nerve impulses. According to Gordon et al. (2004), it also has an impact on the contractility of smooth, skeletal, and cardiac muscle. The potassium levels observed in this investigation were determined to exceed the range of 23.92 to 27.95 parts per million (ppm) reported by Ibidapo et al. (2020) for bread made from a combination of wheat malted millet (Pennisetum Glaucum) and Okara flour blends.The observed high value in this study could potentially be attributed to the inclusion of seed coverings. According to the findings of Tan et al. (2020), legume seed coverings have a high concentration of minerals.

The optimised bread samples and control had zinc concentrations ranging from 1.19 to 1.66 parts per million (ppm). The zinc content of the various bread samples did not exhibit a statistically significant difference ($p>0.05$). The findings of the study indicate that the incorporation of African yam bean seed coat flour into wheat produced bread did not yield a statistically significant ($p>0.05$) impact on the zinc content. Zinc, a vital micronutrient, is present in a wide range of dietary sources and easily accessible water. It is commonly found in the form of salts or organic complexes (Ogunlana et al., 2015). According to Ukom et al. (2019), zinc is considered to have the least hazardous effects compared to other minerals. Insufficient intake of zinc in one's diet can lead to many health issues, including diarrhoea, irritability, and hair loss. The zinc levels observed in the current study are within the range of 1.28 to 2.02 parts per million (ppm) as reported by Bulut (2022) in their study on the mineral composition of various bread wheat cultivars.

The optimised bread samples and control had sodium concentrations ranging from 64.21 to 76.30 ppm. The bread samples containing composite flour exhibited lower sodium values. Specifically, the African yam bean seed coat composite bread demonstrated the lowest sodium value of 64.21 ppm, whereas the 100% wheat bread displayed the highest sodium value of 76.30 ppm. The results of the current study align with the findings given by George et al. (2020), which indicated that African yam bean possesses high levels of magnesium, calcium, and zinc, while exhibiting low levels of sodium and copper. The findings of the study indicate that the incorporation of African yam bean seed coat flours into wheat during the manufacturing of bread resulted in a significant reduction ($p<0.05$) in sodium content. This implies that bread made from wheat flour may contain more sodium compared to bread made from mixed flours made from African yam bean seed coats. Sodium serves as the primary positively charged ion (cation) within the extracellular fluid and plays a crucial role in the retention of bodily fluids. Additionally, it aids in maintaining the body's fluid equilibrium, both internally and externally (Gordon et al., 2004). Nevertheless, Jacobson et al. (2013) found that the use of sodium from processed and restaurant foods is associated with elevated levels of hypertension, myocardial infarction, and cerebrovascular accident.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

The study suggests that individuals, particularly those with hypertension, should restrict their daily sodium intake to 1,500 mg.The sodium levels observed in this investigation were determined to exceed the range of 12.62-13.83 parts per million (ppm) reported by Ibidapo et al. (2020) for bread made from a combination of wheat malted millet (Pennisetum Glaucum) and Okara flour blends.

Table 2: Mineral Composition of Bread Samples from Optimized Composite Flour from African Yam Bean Seed Coat and Seed Coat and 100% Wheat Flour

Sample	Calcium (ppm)	Phosphorus (ppm)	Potassium (ppm)	Zn (ppm)	Na (ppm)
AYB	$44.28^{a}\pm1.99$	$28.89^{a}\pm1.69$	$65.04^{b}\pm0.00$	$1.66^{a}\pm0.52$	$64.21^{c}\pm0.86$
CW	$37.46^{b}\pm0.49$	$24.16^{b}\pm1.21$	$57.44^{c}\pm1.71$	$1.50^{a}\pm0.28$	$76.30^{a}\pm0.93$

Values are means ± standard deviation of duplicate determinations. Means with different superscripts along the same column are significantly different ($p<0.05$).
AYB- Bread sample containing 21.762 g of African yam bean seed coats and 78.238 g of Wheat flour
CW- Control made from 100% wheat flour

C. Antinutrient Composition of Bread Samples from Optimized Composite Flour

The bread samples from optimised composite flour and control exhibited trypsin inhibitor levels ranging from 1.06 to 5.34 %. The bread samples derived from composite flour with African yam bean seed coat bread have the highest trypsin inhibitor content, measuring at 5.34 %. Conversely, the bread made entirely from wheat demonstrates the lowest trypsin inhibitor content, measuring at 1.06 %. The inclusion of seed coat flour resulted in statistically significant levels ($p<0.05$) of trypsin inhibitor content in the bread samples. The findings indicate that it is advisable to exercise caution while including African yam bean seed coat flour in order to prevent an excessive elevation in trypsin inhibitor levels. According to AvilésGaxiola et al. (2018), trypsin inhibitors play a significant role as anti-nutritional agents due to their ability to decrease the process of digestion and absorption of dietary proteins. The present study observed trypsin inhibitor concentrations that exceeded the stated value of 2.89% for wheat-soy bread (Goetz, 2012). The bread samples exhibited trypsin inhibitor levels that were within a comparable range (12.07 to 13.99 mg/g) to those reported by Tan et al. (2020) for the flour samples of the composite.

The bread samples obtained from the optimised composite flour and control groups exhibited oxalate values ranging from 0.03 to 0.17 mg/g. The composite bread samples exhibited a reduced concentration of oxalate compared to the 100% wheat flour sample. The potential cause for the low oxalate content in the composite flour may be attributed to the pretreatment of the seed coat. The bread containing 100% wheat had the highest oxalate content, measuring at 0.17 mg/g, whereas the bread made with African yam bean seed coat displayed the lowest oxalate content, measuring at 0.03 mg/g. There were notable disparities ($p<0.05$) in the oxalate levels between the composite bread sample and the control. Sarkiyayi and Agar (2010) have observed that oxalates have the ability to create insoluble salts with mineral components, including zinc, calcium, and iron. This interaction hinders the utilisation of these minerals within the body's system. According to Okombo and Liebman (2010), the oxalate values observed in the bread samples were determined to be below the range of 16.5 to 45.9 mg/g reported for breads made from a blend of wheat, sweet potatoes, and sesame seeds flour.

The bread samples exhibit a variety of phytate levels, spanning from 0.11 to 0.35 mg/g. The control group had decreased phytate readings compared to the composite bread samples. The African yam bean seed coat bread composite bread sample exhibited the greatest phytate value of 0.35 mg/g, whilst the 100% wheat flour sample had the lowest phytate value of 0.11 mg/g. The findings suggest that incorporating pretreatment African yam bean seed coat flour into wheat may lead to an elevation in the phytate level of the resulting bread variant. The rationale behind this observation is not implausible, given seed coats exhibit elevated levels of anti-nutrients despite undergoing pre-treatment. According to Inyang et al. (2019), phytates have the ability to create complexes with proteins and minerals, including calcium, iron, zinc, and magnesium, resulting in their biological inaccessibility. The present investigation observed phytate readings that were lower than the range of 11.95 to 16.89 mg/g reported by Oguntuase et al. (2022) for bread made from wheat-bambara groundnut flour blends.

The bread samples exhibited a variety of tannin concentrations, varying from 0.21 to 1.39 mg/g. The bread made with African yam bean seed coat flour had the highest tannin content, measuring at 1.39 mg/g. In contrast, the bread made with 100% wheat had the lowest tannin content, measuring at 0.21 mg/g. This difference was statistically significant ($p<0.05$) when compared to the tannin content of 1.39 mg/g observed in the African yam bean seed coat composite bread. Sharma et al. (2021) have documented that tannins have been associated with many physiological effects, including the acceleration of blood coagulation, reduction of blood pressure, decrease in serum cholesterol levels, induction of liver necrosis, and modulation of immunoresponses. Nevertheless, excessive use of tannins can lead to adverse consequences like gastric irritation, nausea, emesis, and hepatic impairment (Omar et al., 2022). According to Omar et al. (2022), there appears to be a positive correlation between the regular consumption of foods containing high levels of tannin and an elevated risk of getting nose or throat cancer. The present study observed

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

tannin values that were lower than the range of 1.81 to 3.24 mg/g reported by Oguntuase et al. (2022) for bread samples made from a blend of wheat and bambara groundnut flour. However, these values were higher than the range of 0.06 to 1.02 mg/g reported by Henry-Unaeze and Amadi (2022) for bread made from a blend of wheat, African yam bean and corn seed flour. Nevertheless, these tannin values fall within the recommended safe range for legumes, which is between 0.3 a Tannins, which are anti-nutritional compounds, are found mostly in the seed coat or testa of dry beans (Sorour et al., 2018).

Table 3: Anti-Nutrient Content of Bread Samples from Optimized Composite Flour from African Yam Bean Seed Coat and 100% Wheat Flour

Sample	Trypsin Inhibitor (%)	Oxalate (mg/g)	Phytate (mg/g)	Tannin (mg/g)
AYB	5.34^{a}±0.07	0.03^{b}±0.05	0.35^{b}±0.41	1.39^{b}±0.02
CW	1.06^{b}±0.04	0.17^{a}±0.09	0.11^{a}±0.41	0.21^{a}±0.14

Values are means ± standard deviation of duplicate determinations. Means with different superscripts along the same column are significantly different (p<0.05).

AYB- Bread sample containing 21.762 g of African yam bean seed coats and 78.238 g of Wheat flour

CW- Control made from 100% wheat flour

E. Descriptive Sensory Test of Bread from Optimized Composite Flour Blend (African Yam Bean Seed Coat) and 100% Wheat Flour

Table 4 displays the outcomes of the descriptive analysis. The mean scores for the look of the control and optimised samples ranged from 7.81 to 6.35, respectively. A statistically significant difference (P<0.05) was observed across the samples. The bread samples exhibited a range of crust colour and varying degrees of colour darkness, spanning from light to dark brown. The bread sample labelled as 506, which was made using a composite flour derived from African yam bean seed coat, had the most intense crust coloration, which can be attributed to the presence of seed coats within the bread.

The texture of the bread samples varied between 7.56% and 6.24%. A notable disparity (p<0.05) was observed across the bread samples. The texture attribute refers to the bread's capacity to withstand the pressure exerted by the crumb on the finger and its ability to revert back to its initial position following compression. The texture feature of Sample 732's 100% wheat flour is the most favourable. The bread samples had odours ranging from 5.77 to 5.03, respectively. A notable disparity (p<0.05) was observed between the control and composite bread samples. The pleasant fragrance of the bread may be attributed to the seed coatings present in the flour, which are detected through the orthonasal channel. This perception affects the preference for bread made from African yam bean flour compared to bread made from 100% wheat flour.

Table 4: Descriptive Mean Score for Sensory Attributes of Bread made from Optimized Composite Flour from African Yam Bean, Bambara Groundnut Seed Coat and Wheat Flour.

	Samples	
Attribute	**506**	**732**
Appearance	6.35^{c} ± 176	7.81^{a} ± 1.41
Texture	6.24^{b} ± 1.67	7.53^{a} ± 1.10
Odour	5.77^{a} ± 1.68	5.03^{b} ± 1.49

Values are means ± standard deviation of duplicate determinations. Means with different superscripts along the same column are significantly different (p<0.05).

506 – Composite flour (African yam bean seed coat) and 732 – 100% Wheat flour (Control)

KEY: Hedonic Scale: 1- Dislike Extremely; 2 – Dislike Much; 3 - Dislike Moderately; 4 - Dislike slightly; 5 - Neither like nor dislike; 6 - Like slightly; 7 – Like moderately; 8 – Like very much; 9 – Like extremely

IV. CONCLUSION

The study revealed that inclusion of pretreated seed coat of African yam bean in the production of bread caused an increase in the antioxidant properties in terms of total phenols, ABTS and total carotenoids. There was also improvement in the minerals like calcium, phosphorus, potassium and zinc, however, there was reduction in the sodium content. Anti-nutritional factors were also reduced. In conclusion, the sensory assessment of the bread indicated that sample 506, which is made from a composite flour derived from the seed coat of African yam beans, met the acceptable standards.

AUTHORS' CONTRIBUTIONS

OOL conducted the experiments and analysis, AAF designed the study and was responsible for the revision of the manuscript. All authors read and approved the final manuscript.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR1595

Authors' declaration on competing interest: The authors wish to state that there is no competing interest whether financially or otherwise.

ACKNOWLEDGEMENTS

The following people are greatly appreciated for their contributions to the success of the research, Tertiary Education Trust Fund (TETFund); Professor Adeoluwa, V.O., The Vice Chancellor of Bamidele Olumilua University of Education, Science and Technology, Ikere Ekiti and Centre for Research and Development (CERAD), Bamidele Olumilua University of Education, Science and Technology, Ikere Ekiti.

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